Figure 1

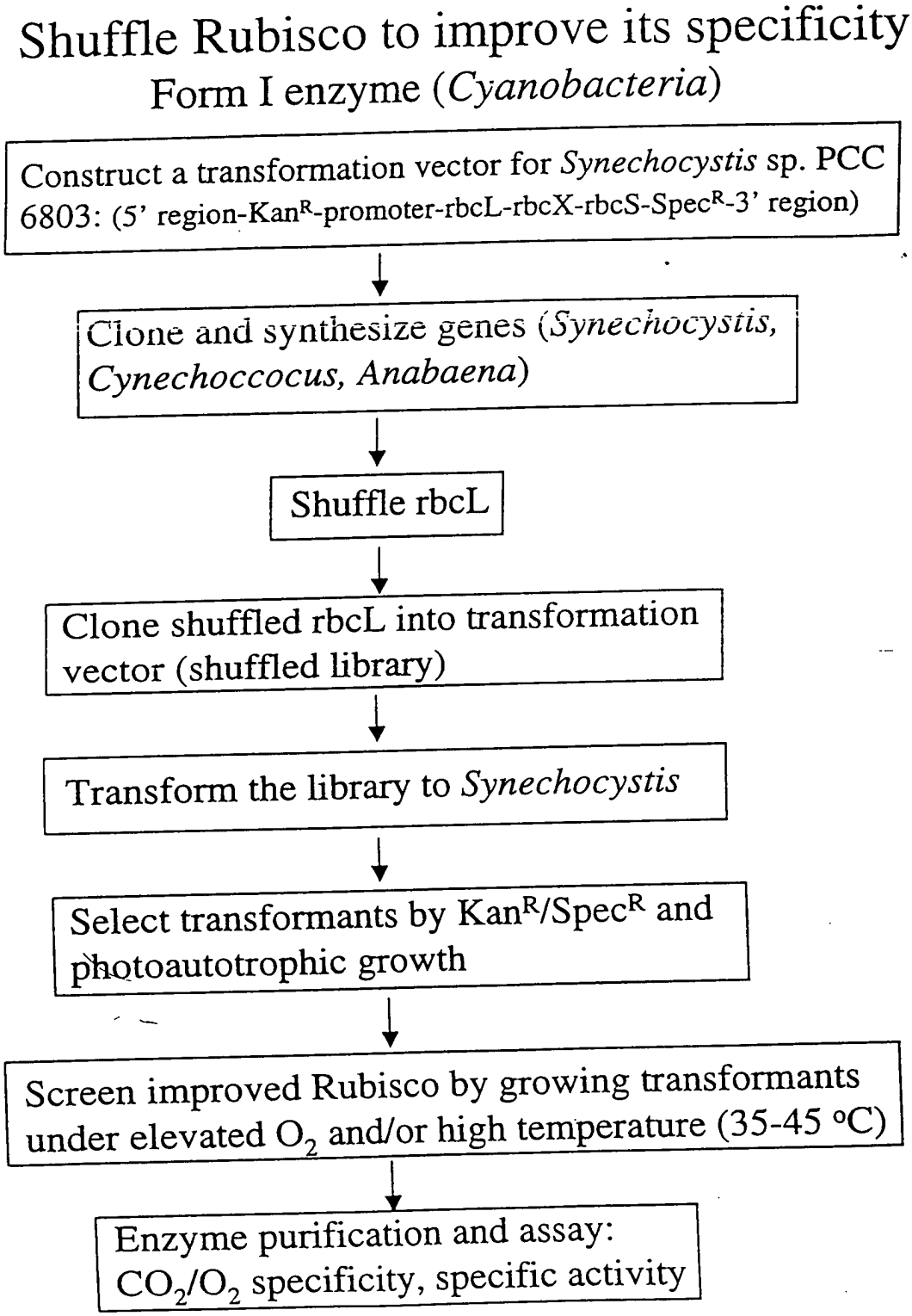
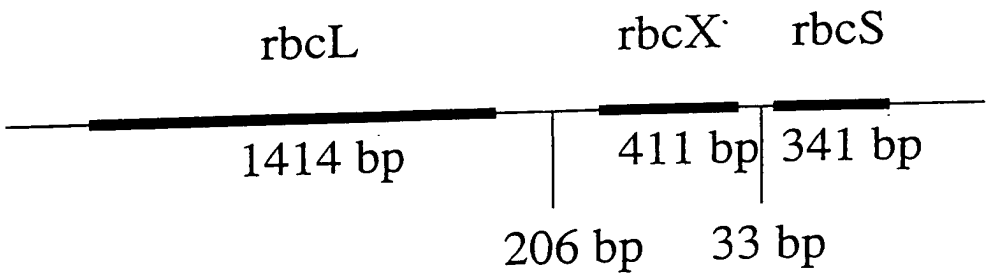

Shuffle Rubisco to improve its specificity
Form I enzyme (*Cyanobacteria*)
Construct a transformation vector for *Synechocystis* sp. PCC 6803: (5' region-Kan^R-promoter-rbcL-rbcX-rbcS-Spec^R-3' region)
Clone and synthesize genes (*Synechocystis, Cynechoccocus, Anabaena*)
Shuffle rbcL
Clone shuffled rbcL into transformation vector (shuffled library)
Transform the library to *Synechocystis*
Select transformants by Kan^R/Spec^R and photoautotrophic growth
Screen improved Rubisco by growing transformants under elevated O_2 and/or high temperature (35-45 °C)
Enzyme purification and assay: CO_2/O_2 specificity, specific activity

Figure 2

A *Synechocystis* Rubisco gene structure



B *Synechocystis* transformation - homologous replacement

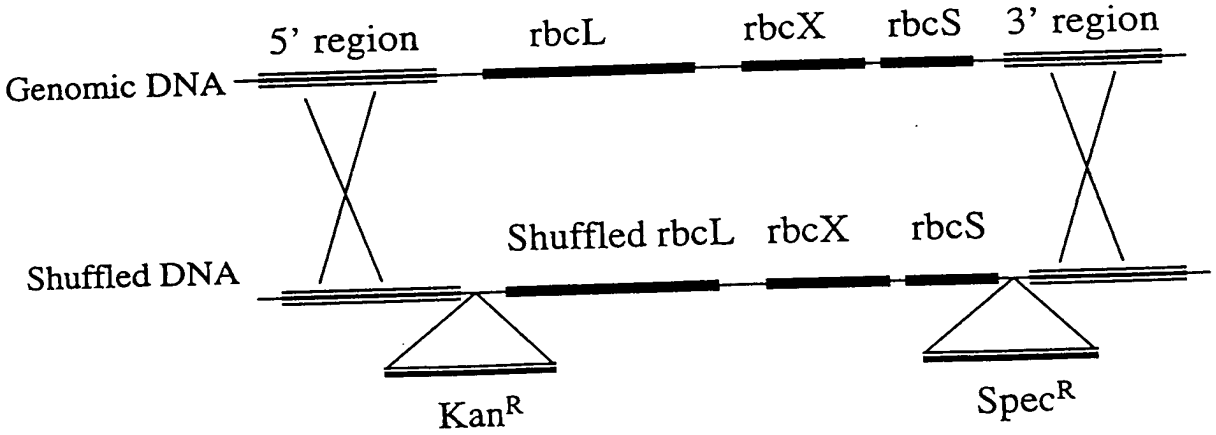


Figure 3

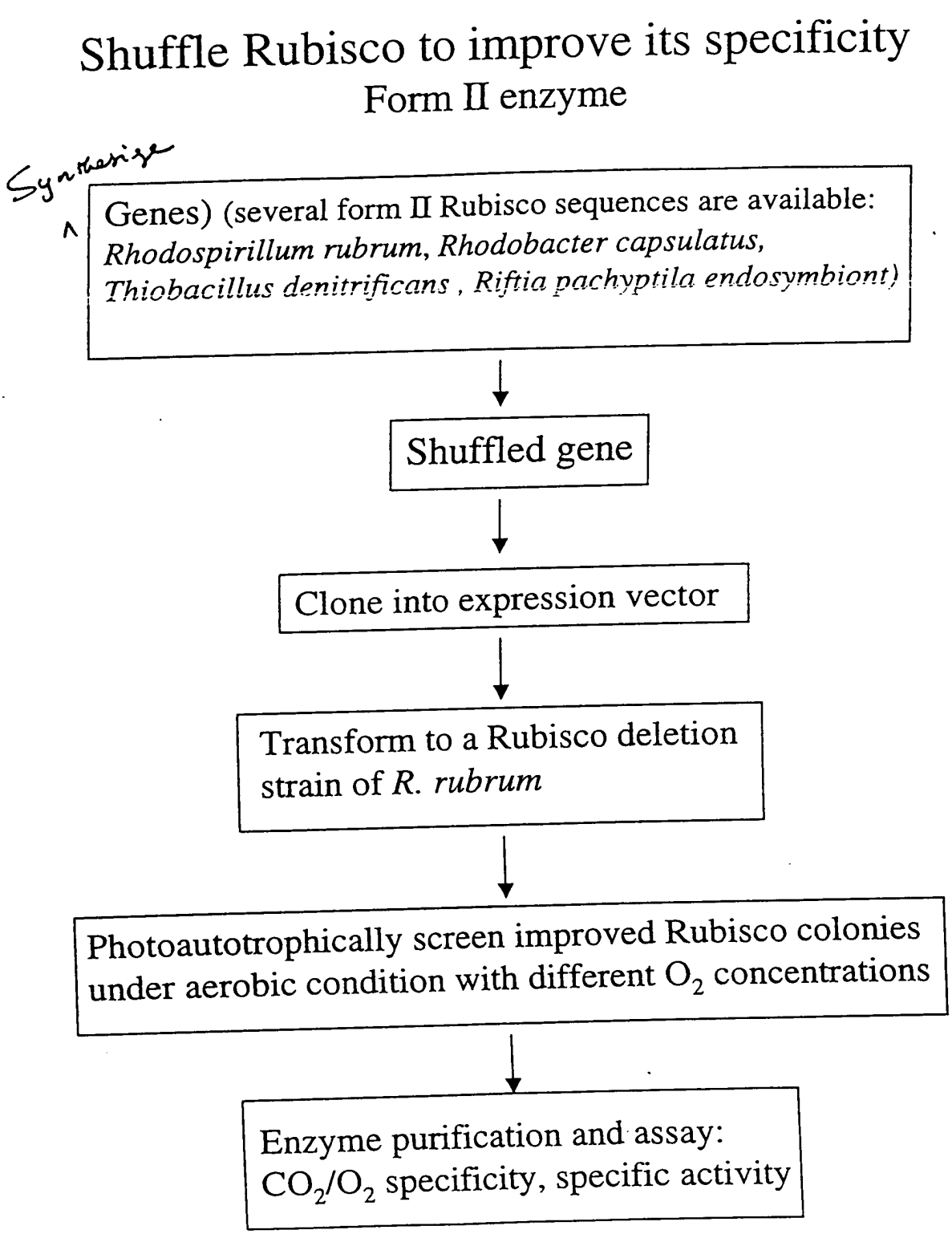

Shuffle Rubisco to improve its specificity
Form II enzyme
Synthesize
Genes) (several form II Rubisco sequences are available: *Rhodospirillum rubrum*, *Rhodobacter capsulatus*, *Thiobacillus denitrificans* , *Riftia pachyptila endosymbiont)*
Shuffled gene
Clone into expression vector
Transform to a Rubisco deletion strain of *R. rubrum*
Photoautotrophically screen improved Rubisco colonies under aerobic condition with different O_2 concentrations
Enzyme purification and assay: CO_2/O_2 specificity, specific activity

Figure 4

Shuffle Rubisco to improve its specificity

Form II enzyme

E. coli screening system (prk)

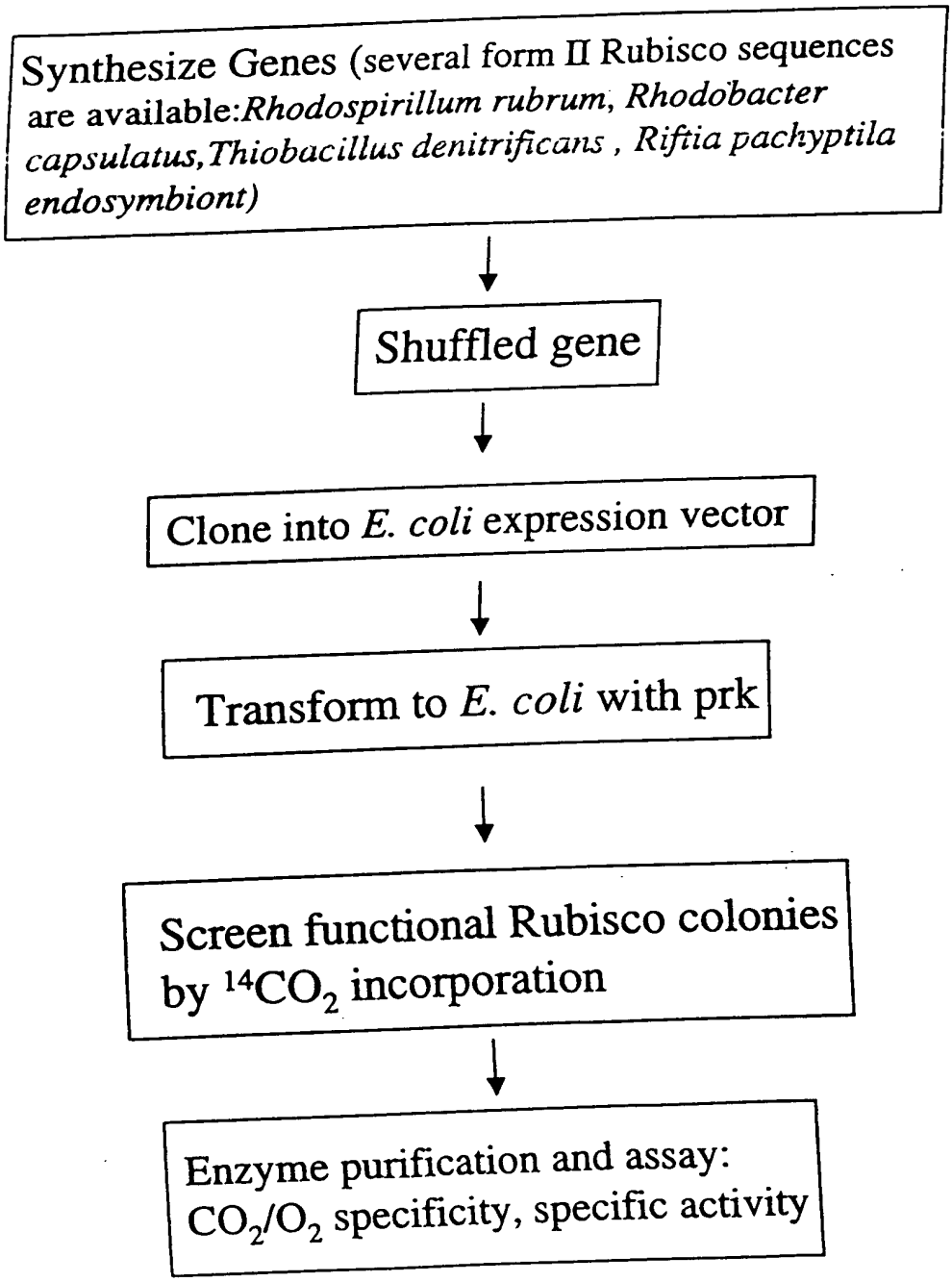
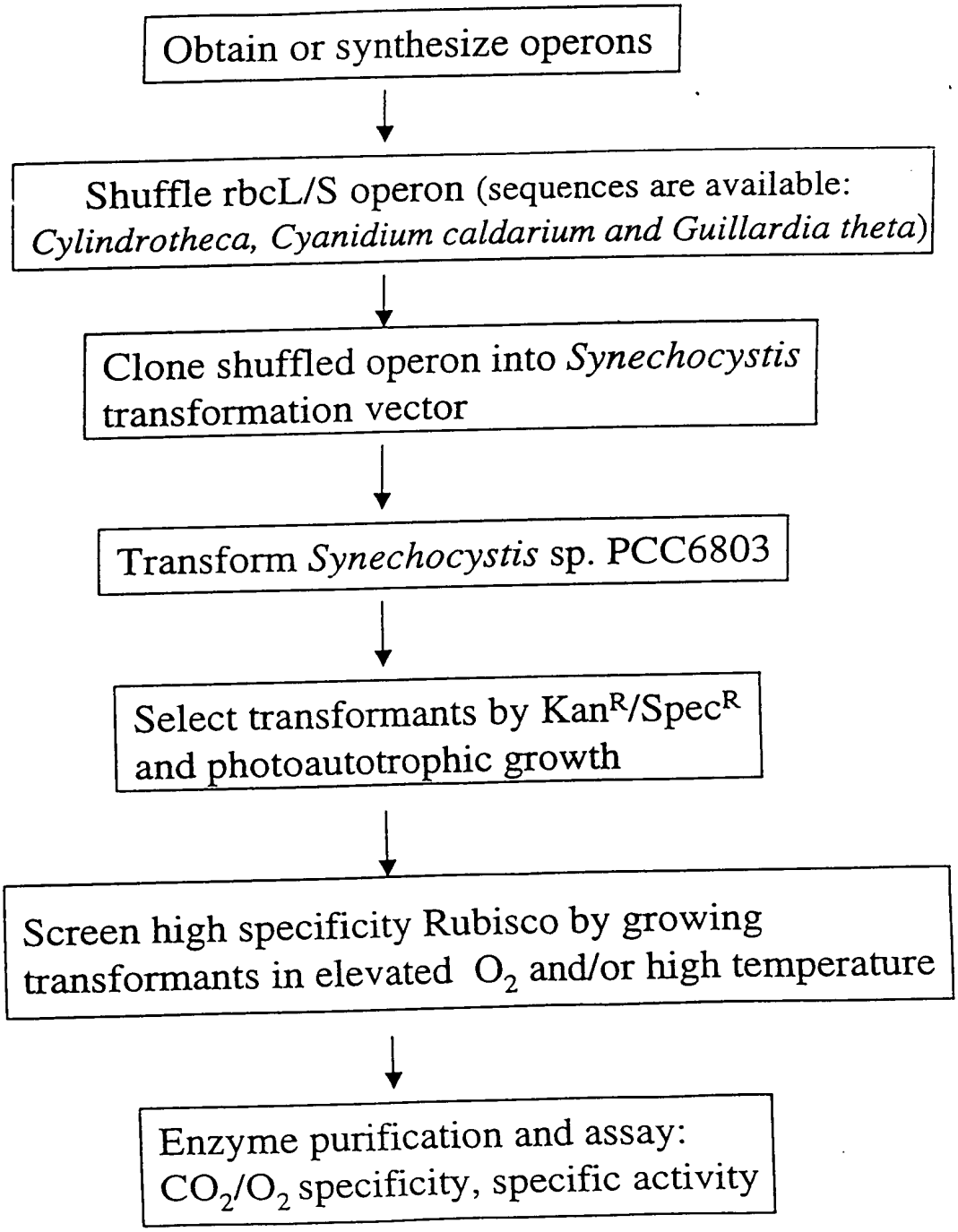


Figure 5


Shuffle high specificity marine Rubisco rbcL/S operon
Obtain or synthesize operons
Shuffle rbcL/S operon (sequences are available: *Cylindrotheca, Cyanidium caldarium and Guillardia theta*)
Clone shuffled operon into *Synechocystis* transformation vector
Transform *Synechocystis* sp. PCC6803
Select transformants by Kan^R/$Spec^R$ and photoautotrophic growth
Screen high specificity Rubisco by growing transformants in elevated O_2 and/or high temperature
Enzyme purification and assay: CO_2/O_2 specificity, specific activity